Supporting Information

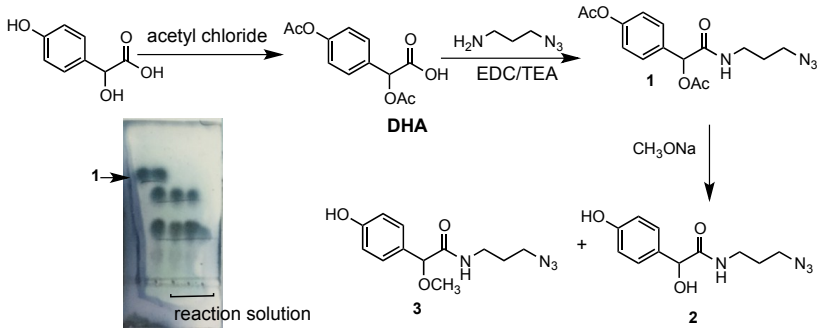
An In Cellulo-Activated Multicolor Cell Labeling Approach Used to Image Dying Cell Clearance

Yilong Shi,[a] Rui Zhu,[a] Zhongwei Xue,[a] Jiahuai Han,[b] and Shoufa Han[a,*]

[a]State Key Laboratory for Physical Chemistry of Solid Surfaces, Department of Chemical Biology, College of Chemistry and Chemical Engineering, the Key Laboratory for Chemical Biology of Fujian Province, The MOE Key Laboratory of Spectrochemical Analysis & Instrumentation, and Innovation Center for Cell Signalling Network, Xiamen University;

[b]State key Laboratory of Cellular Stress Biology, Innovation Center for Cell Signalling Network, School of Life Sciences, Xiamen University, Xiamen, 361005, China; Tel: 86-0592-2181728; E-mail: shoufa@xmu.edu.cn.

Content



Scheme S1. Synthesis and chemical deacetylation of compound **1**.

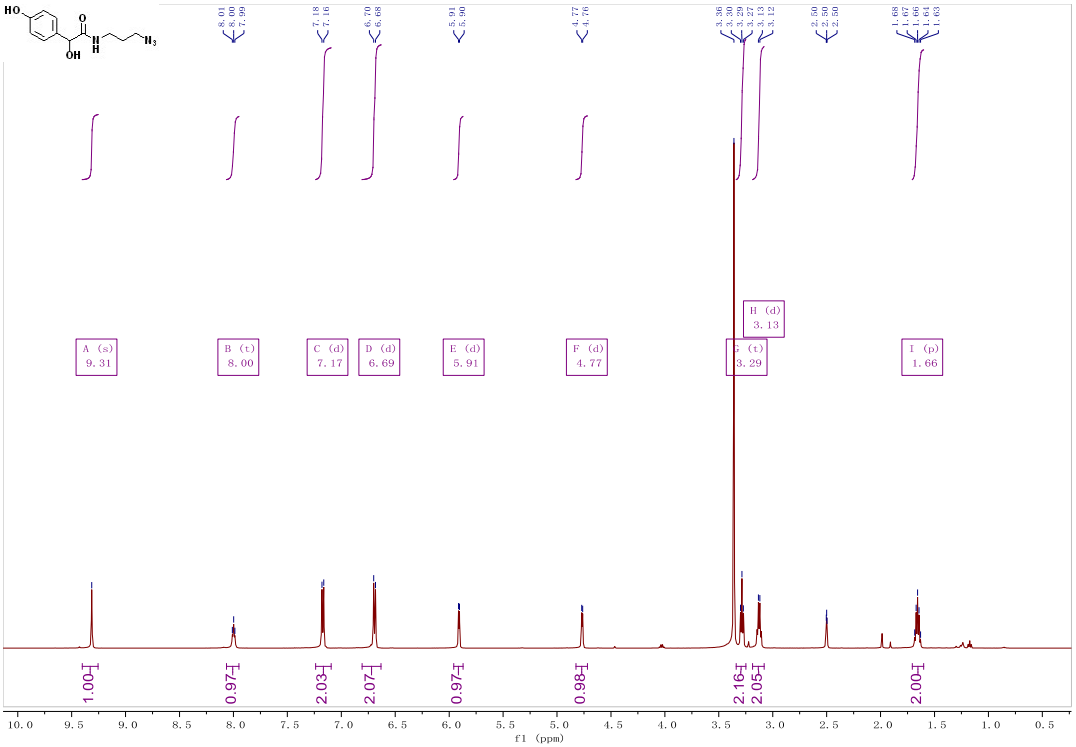


Fig. S1 ^{1}H-NMR of compound **2**

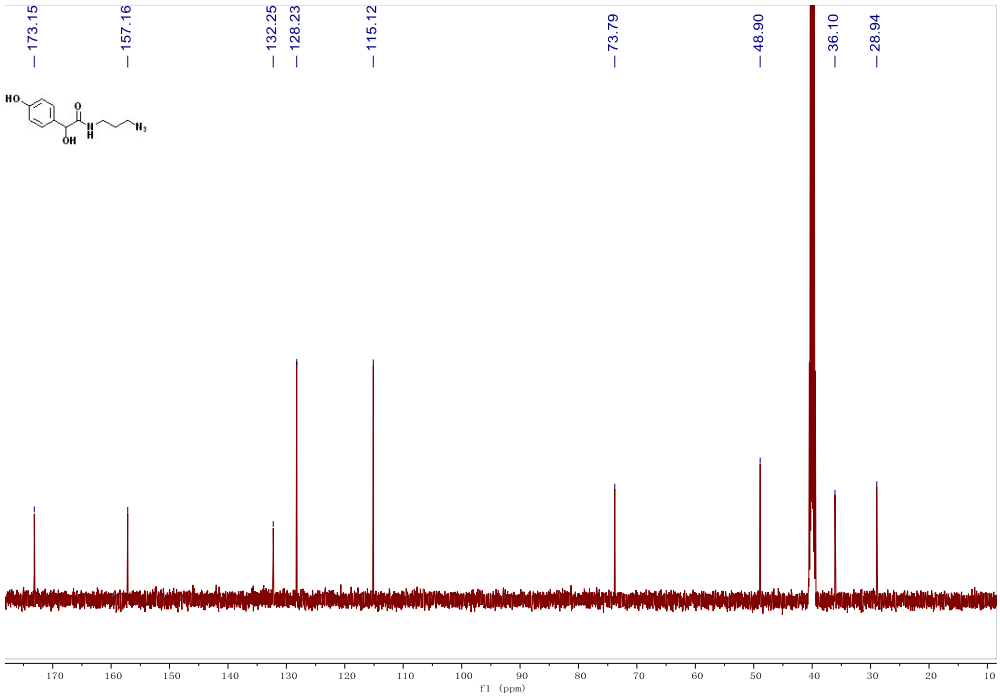


Fig. S2 ^{13}C-NMR of compound **2**

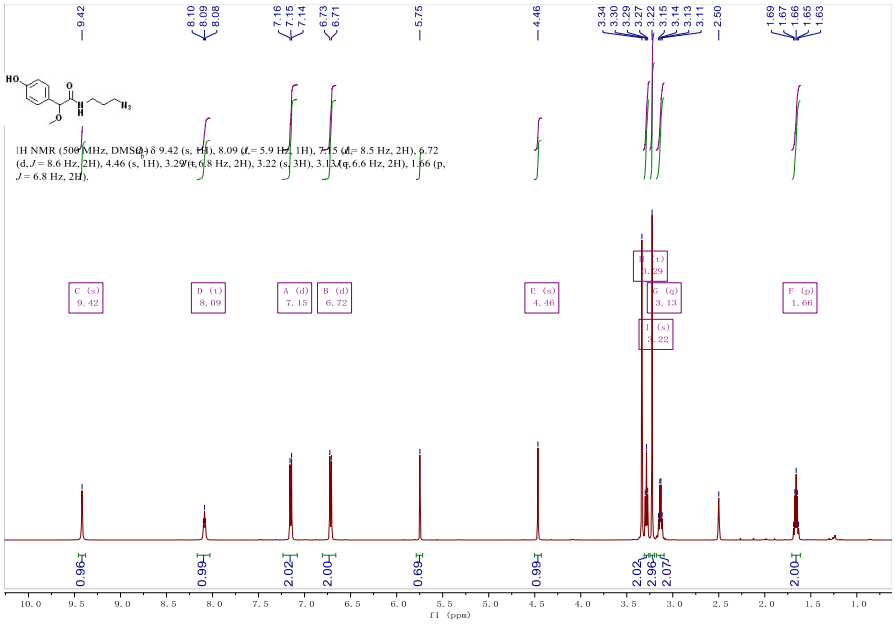


Fig. S3 ^{1}H-NMR of compound **3**

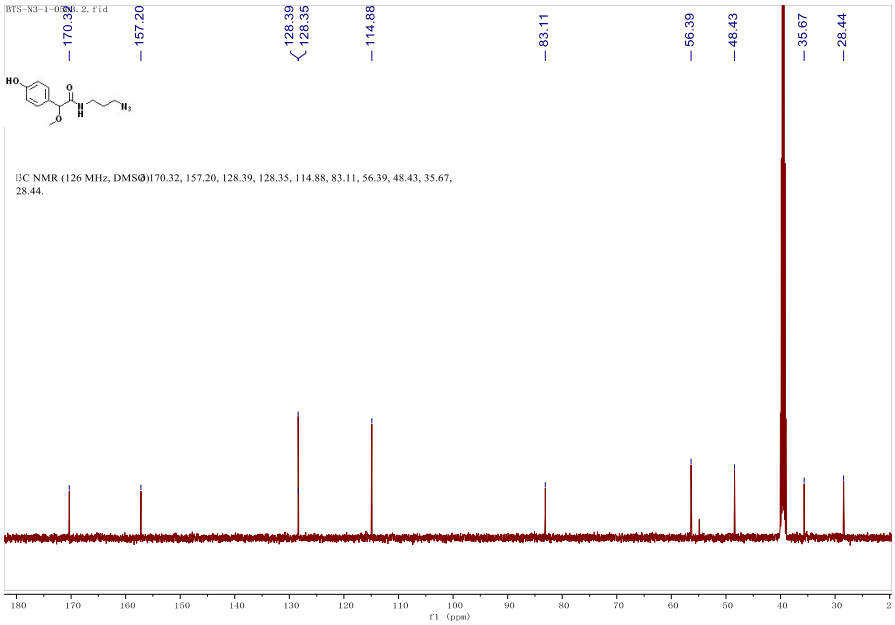


Fig. S4 ^{13}C-NMR of compound **3**

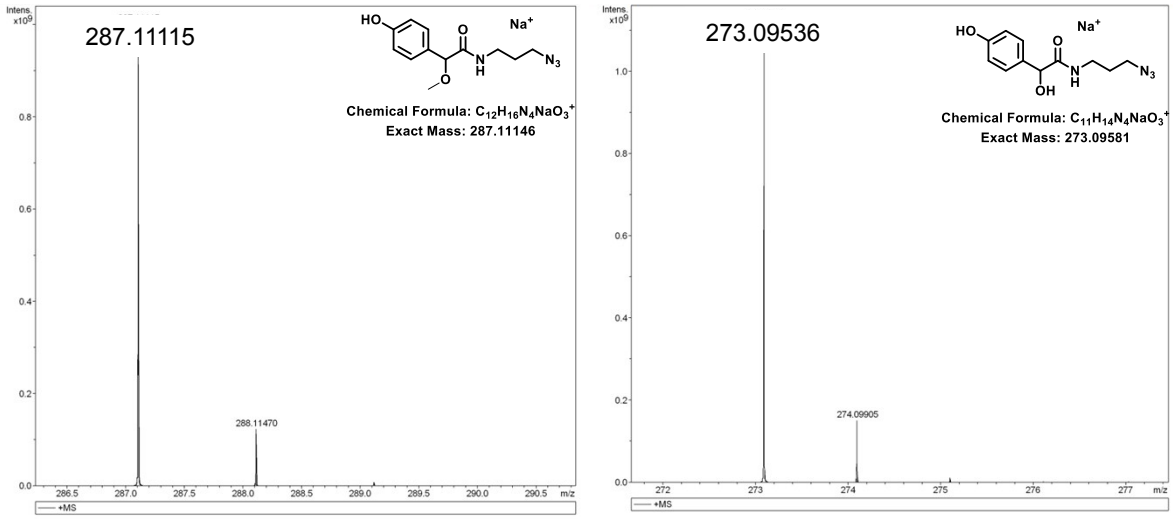
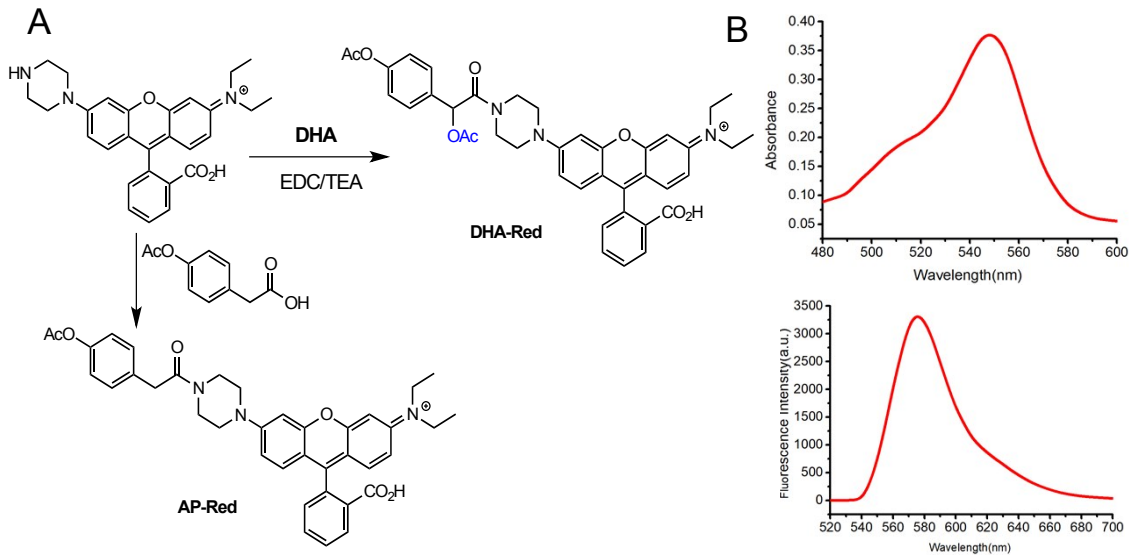
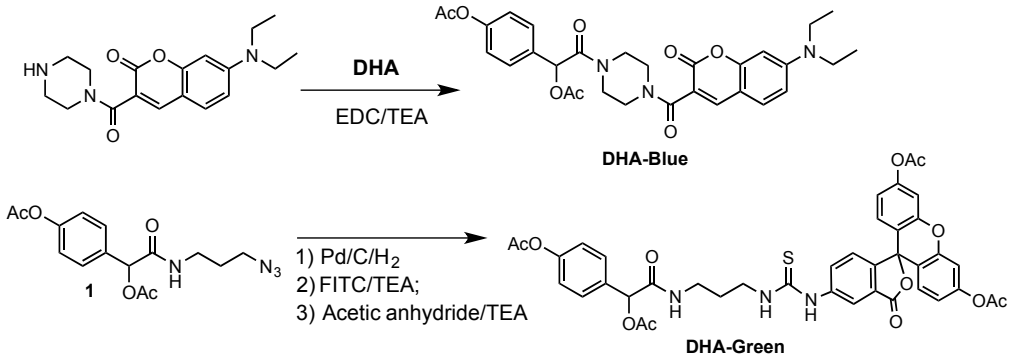


Fig. S5 HRMS of compound **2** and compound **3**



Scheme S2. Synthetic routes for DHA-Red and AP-Red (A). Fluorescence properties of DHA-Red (4 μM) in PBS (pH 7.2) (B)



Scheme S3. Synthetic routes for DHA-Blue and DHA-Green.

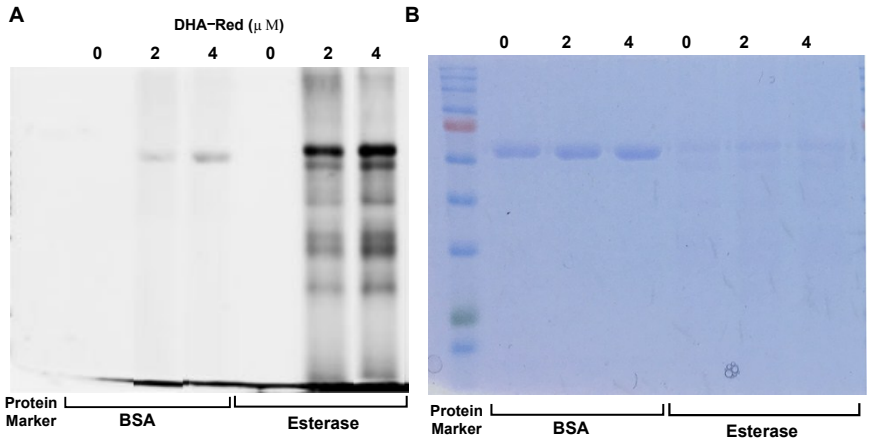


Fig. S6 Esterase-triggered protein labeling with DHA-Red**.** Esterase or BSA (1.5 μg) were treated DHA-Red for 1 h and then subjected to SDS-PAGE. The gel was analyzed for fluorescence emission using λ_{ex} = 520 nm (A) or stained with Coomassie Brilliant Blue (B).

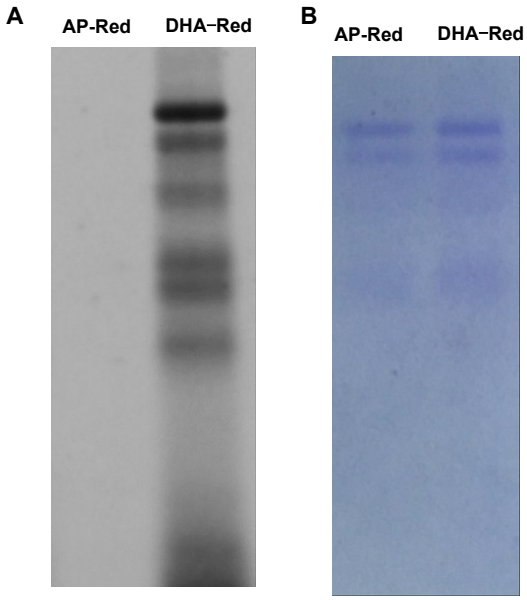


Fig. S7 Incapability of AP-Red to label cellular proteins

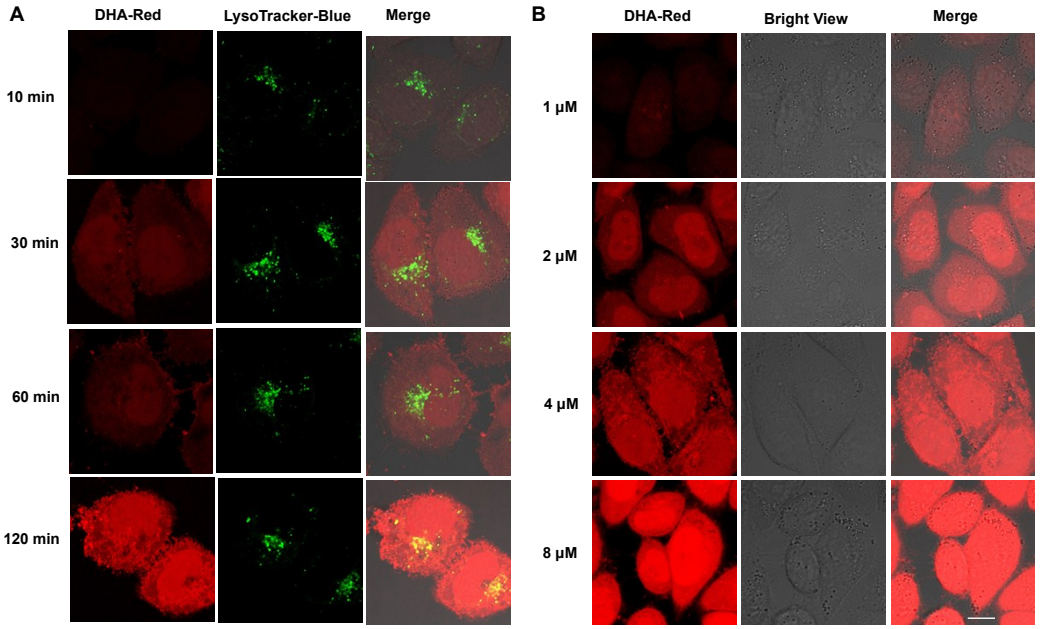


Fig. S8 Dose- and Incubation time-dependent cell labelling with DHA-Red. (A) HeLa cells were co-stained with LysoTracker Blue (1 μM) and DHA-Green (2 μM) for 10, 30, 60, 120 min in DMEM and then imaged by confocal microscopy for intracellular fluorescence. (B) HeLa cells labeled with DHA-Green (1, 2, 4, 8 μM) for 60 min and then imaged by confocal microscopy for intracellular fluorescence. Scale bar: 10 μm.

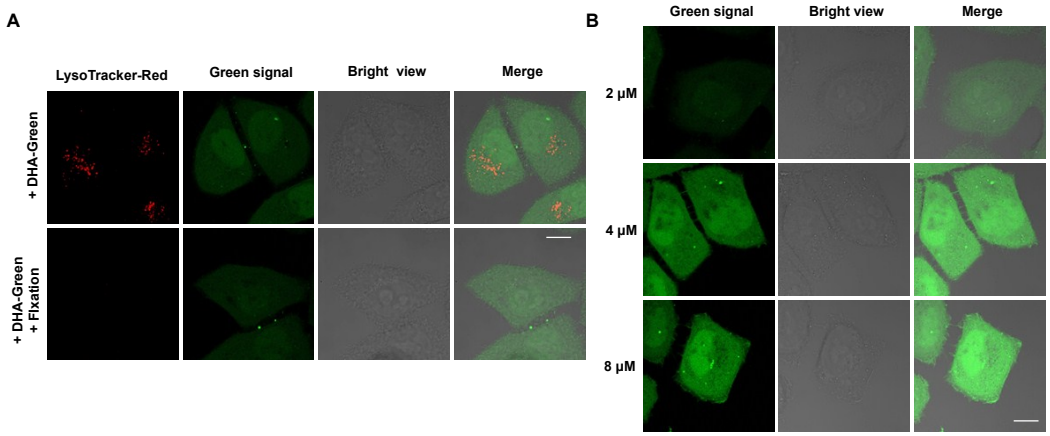


Fig. S9 Covalent cell labelling with DHA-Green. (A) HeLa cells were co-stained with LysoTracker Red (1 μM) and DHA-Green (4 μM) for 1 h. Cells were fixed with 4% Paraformaldehyde Fix Solution, washed and then imaged for intracellular fluorescence. (B) HeLa cells labeled with DHA-Green (2, 4, 8 μM) for 1 h and then fixed before confocal microscopy analysis. Scale bar: 10 μm.

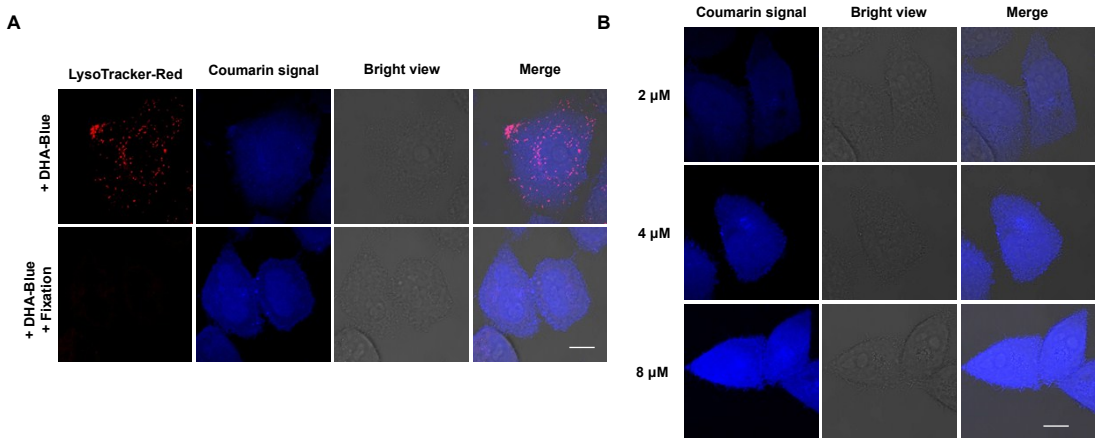


Fig. S10 Covalent cell labelling with DHA-Blue. (A) HeLa cells were co-stained with LysoTracker Red (1 μM) and DHA-Blue (4 μM) for 1 h. The cells were fixed with 4% paraformaldehyde Fix Solution, washed and then imaged for intracellular fluorescence. (B) HeLa cells labeled with DHA-Blue (2, 4, 8 μM) for 1 h and then fixed before confocal microscopy analysis. Scale bar: 10 μm

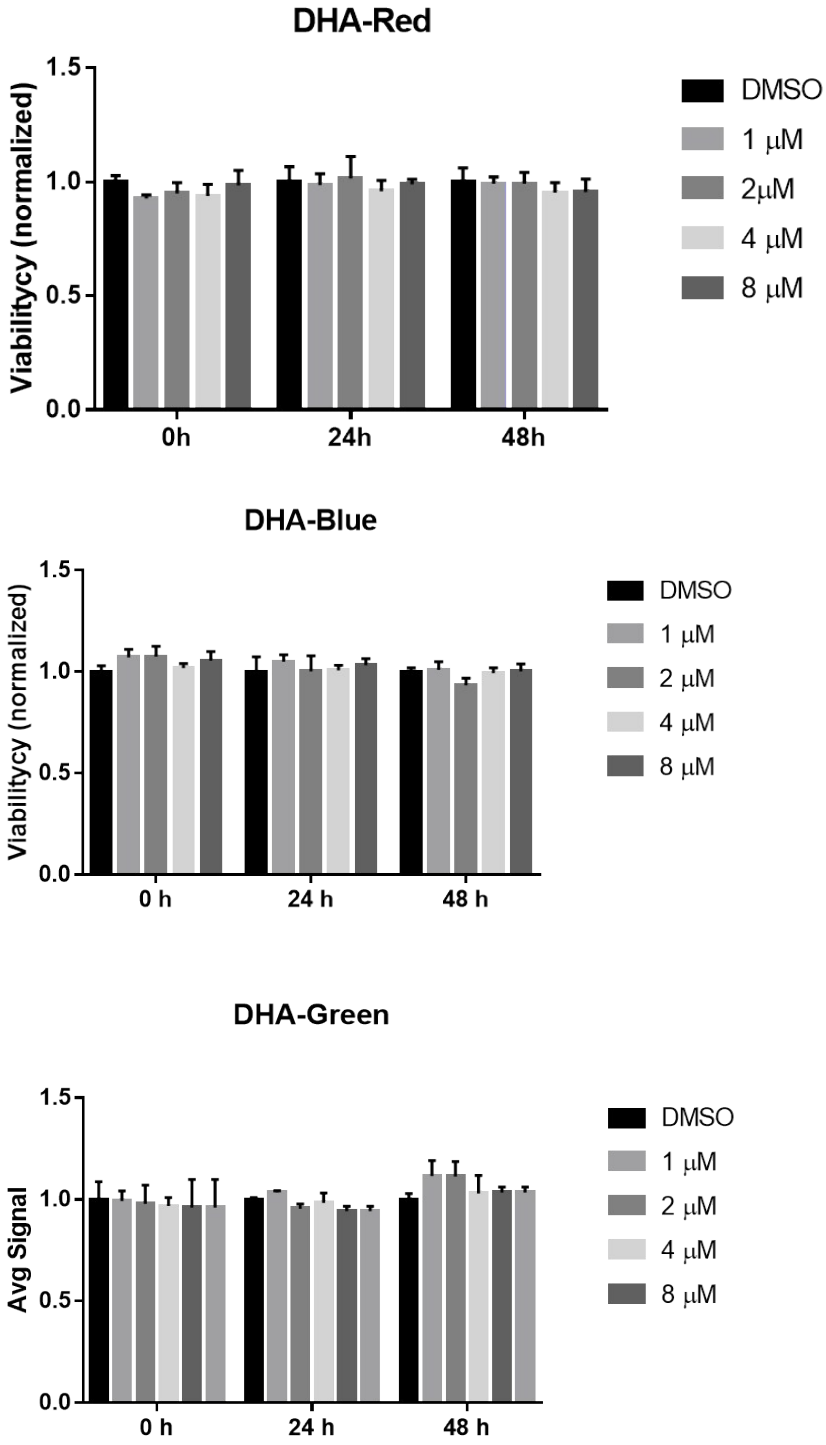


Fig. S11 cytotoxicity of Dye-DHA diads.

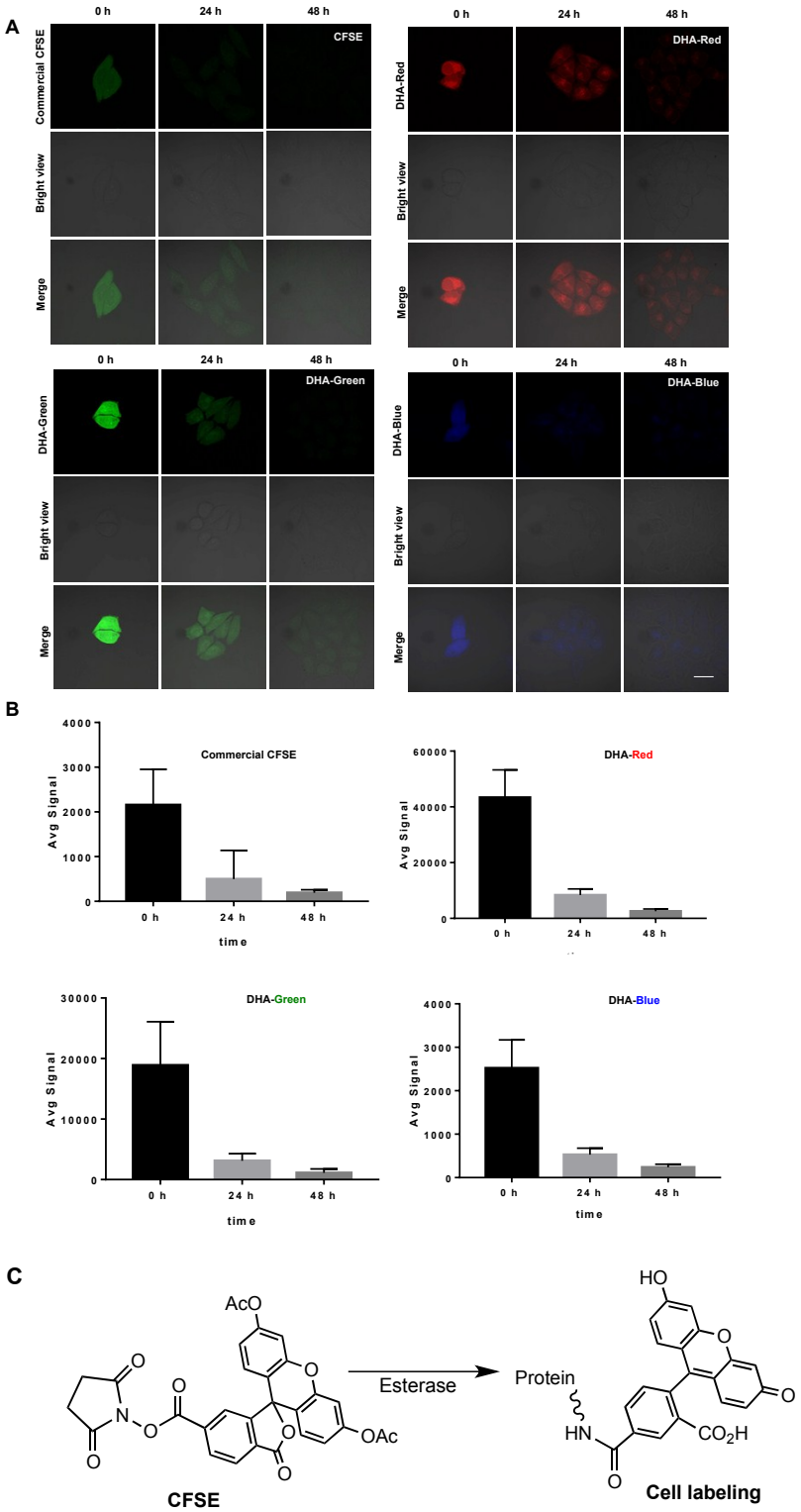


Fig. S12 Comparison of DHA-Dye diads to Cell-tracker CFSE. HeLa cells were stained with CFSE (4 μM), DHA-Blue (4 μM), DHA-Green (4 μM), or DHA-Red (4 μM) for 60 min, respectively. The cells were further maintained in fresh DMEM for 0, 24, 48 h. A portion of the cells were harvested at indicated time points, rinsed with DMEM, and then analyzed by confocal microscopy (A) or flow cytometry (B) for intracellular fluorescence. (B) Chemistry of DHA-Green deacetylation in live cells. Scale bar: 10 μm.

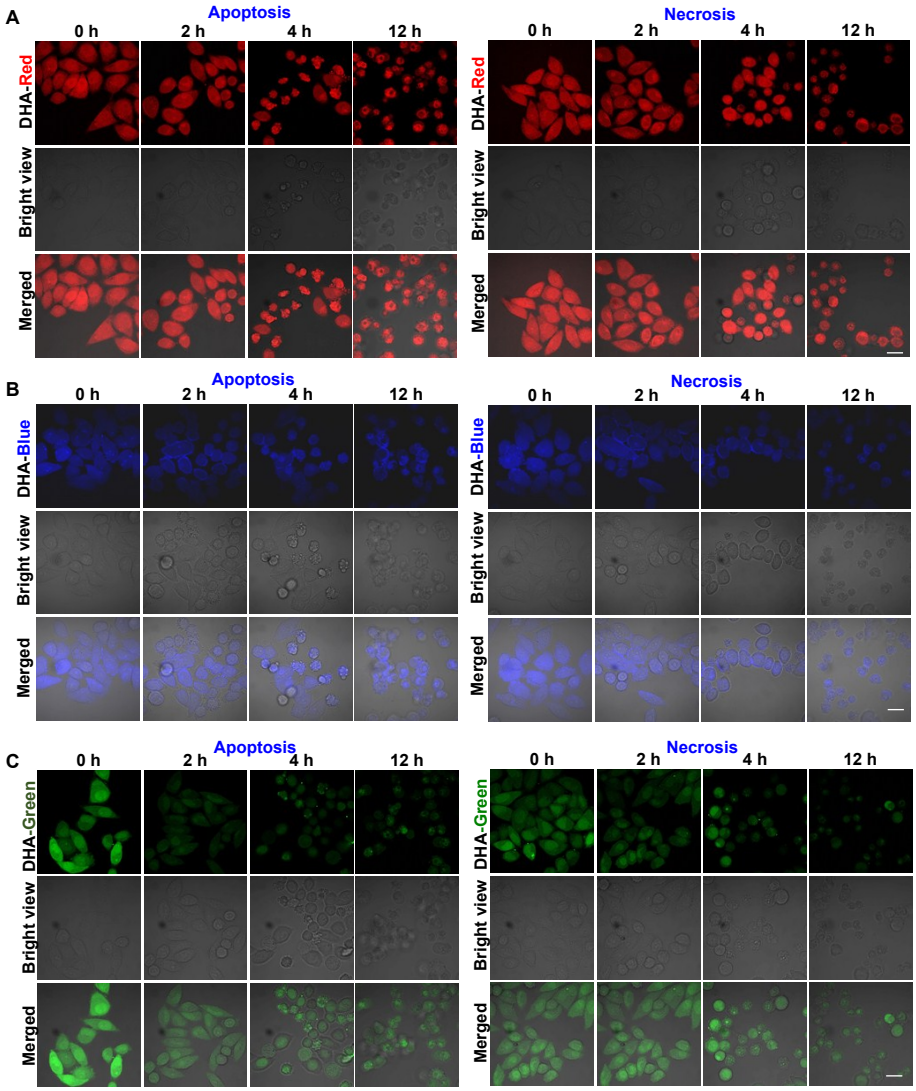


Fig. S13 Fluorescence retention of DHA-medaited labeling in HeLa cells undergoing cell death. RIP3^{+} HeLa cells were stained with DHA-Blue (4 μM), DHA-Red (4 μM), or DHA-Green (4 μM) for 1 h, and then rinsced with PBS, and then treated with Smac/TNF to triggered apoptosis or with Smac/TNF/Z-VAD to trigger necrosis. The dying cells were maintained in fresh medium and then monitored by confocal fluorescence microscopy for intracellualr fluorescence over time. Scale bars: 20 μm.

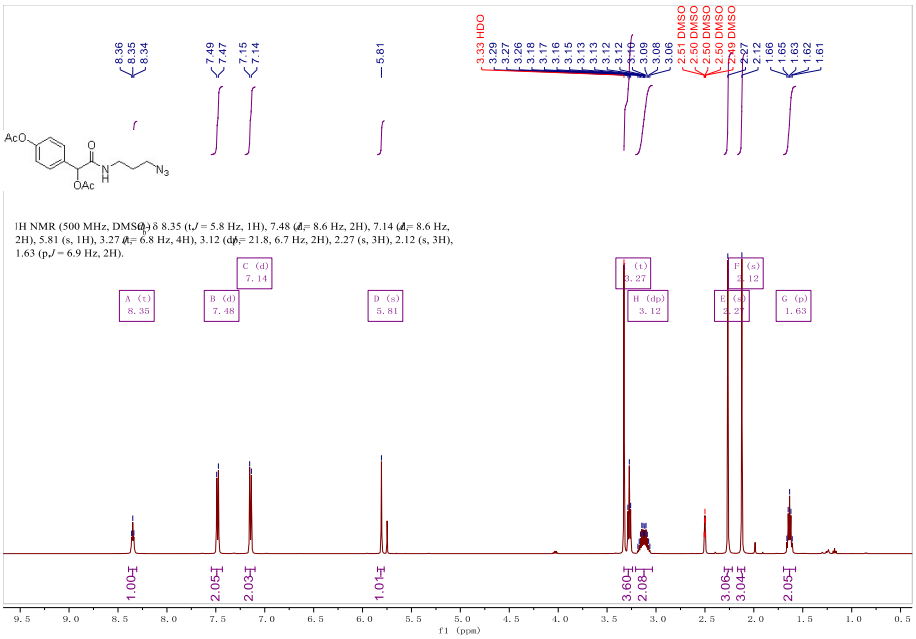


Fig. S14 ^{1}H-NMR of compound **1**

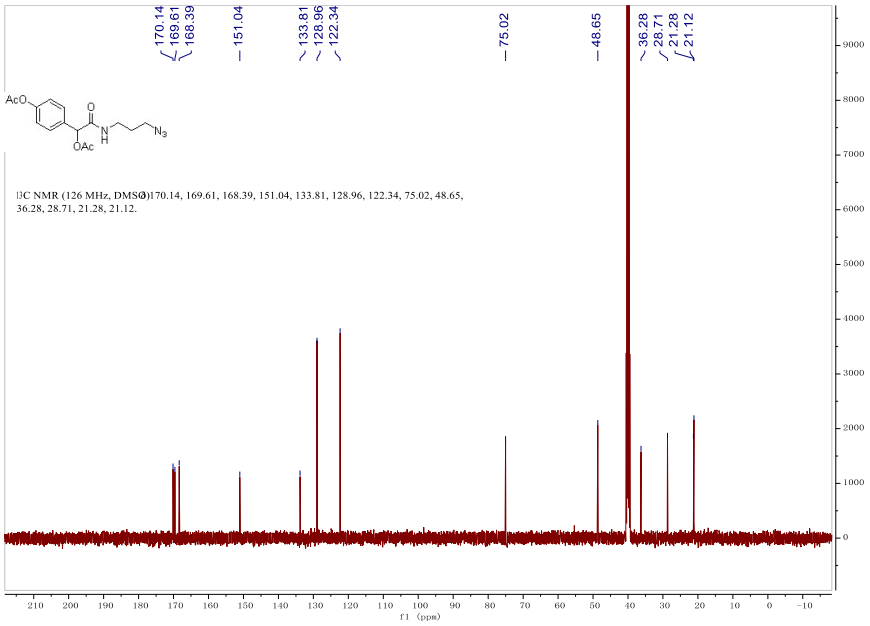


Fig. S15 ^{13}C-NMR of compound **1**

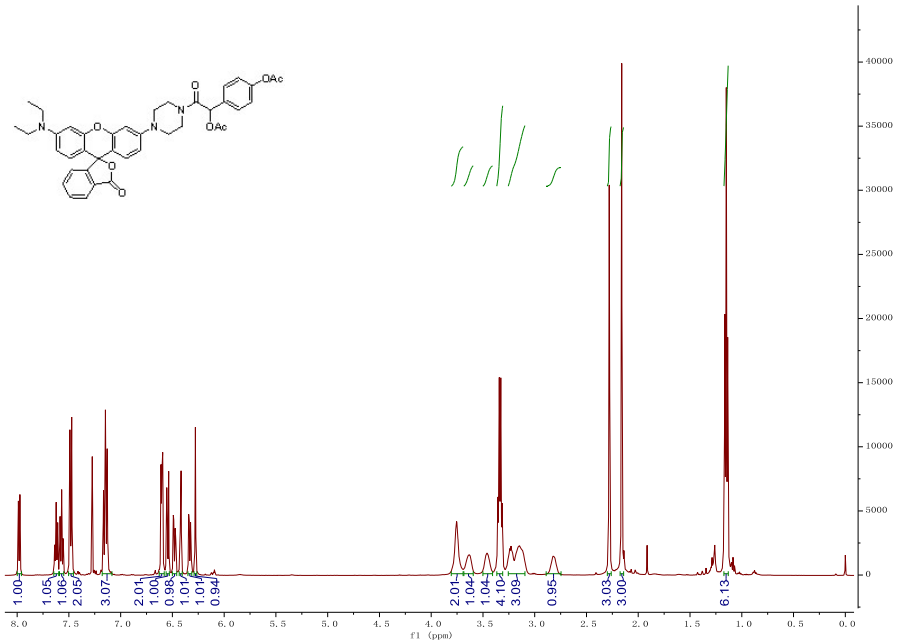


Fig. S16 ^{1}H-NMR of DHA-Red

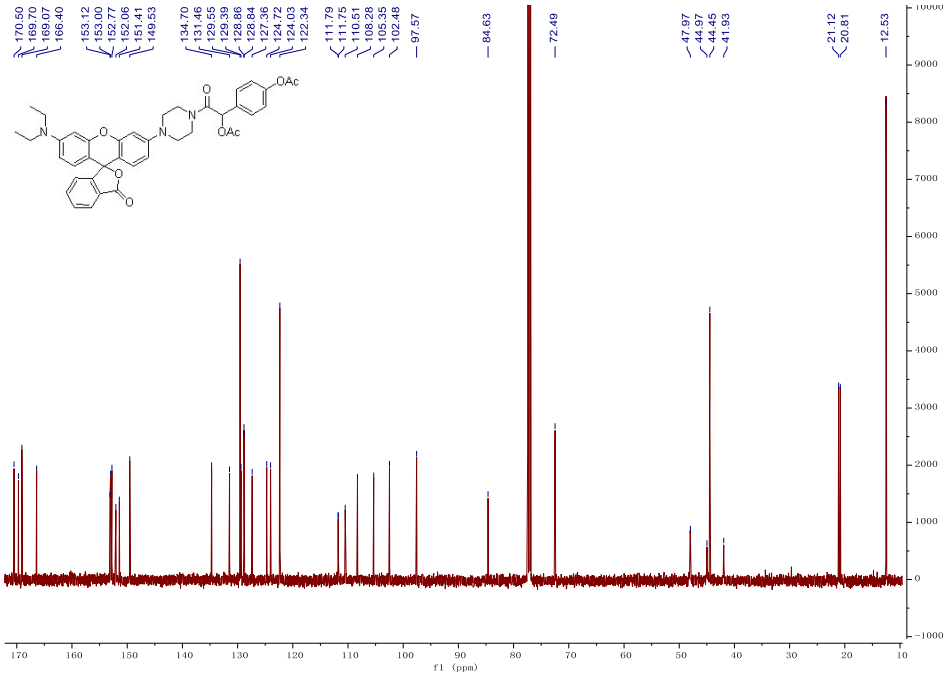


Fig. S17 ^{13}C-NMR of DHA-Red

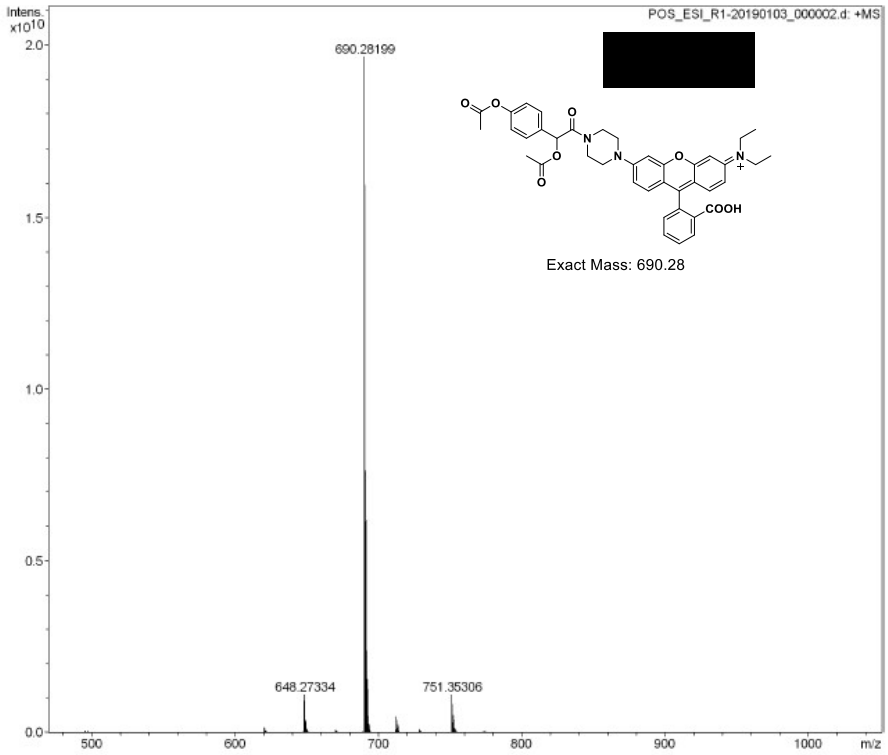


Fig. S18 HRMS of DHA-Red

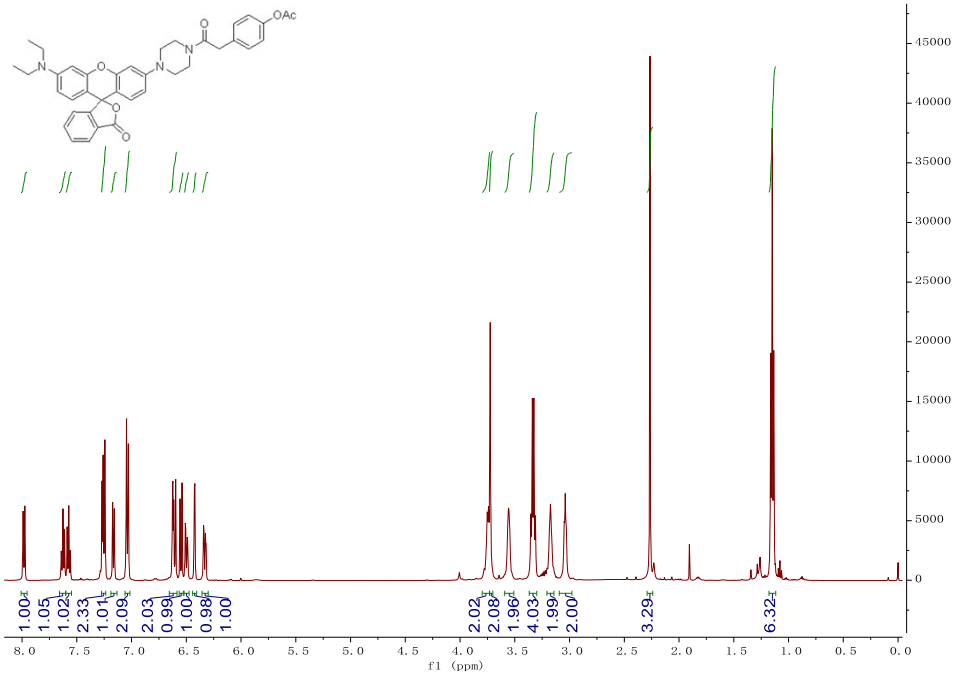


Fig. S19 ^{1}H-NMR of AP-Red

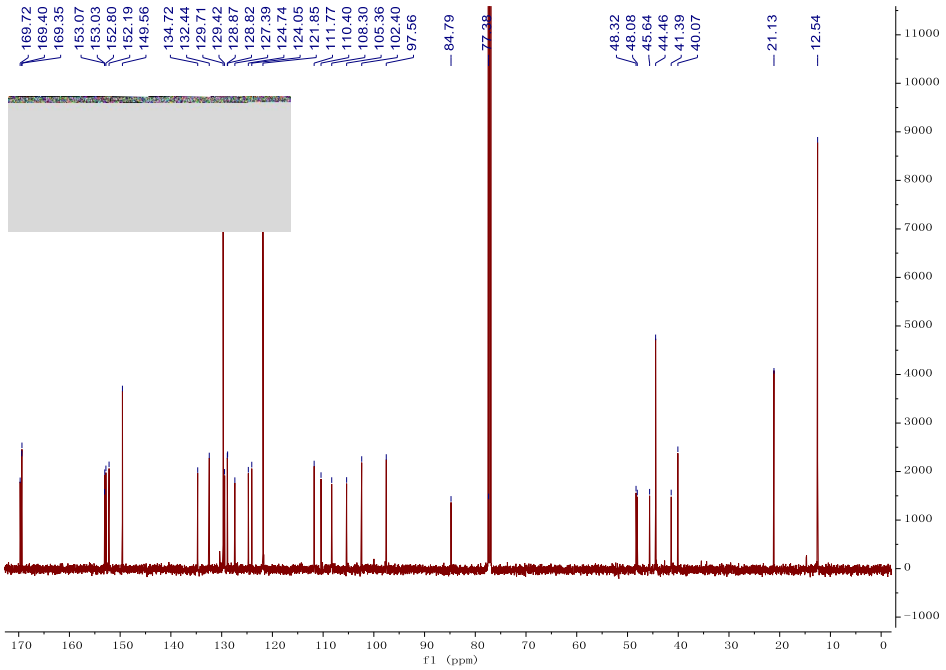


Fig. S20 ^{13}C-NMR of AP-Red

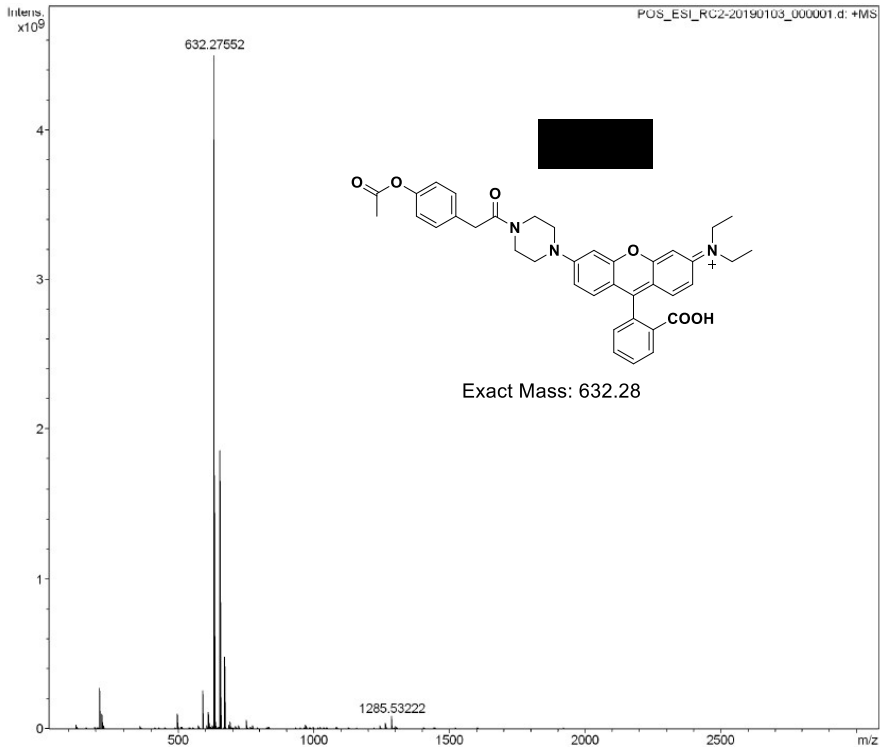


Fig. S21 HRMS of AP-Red

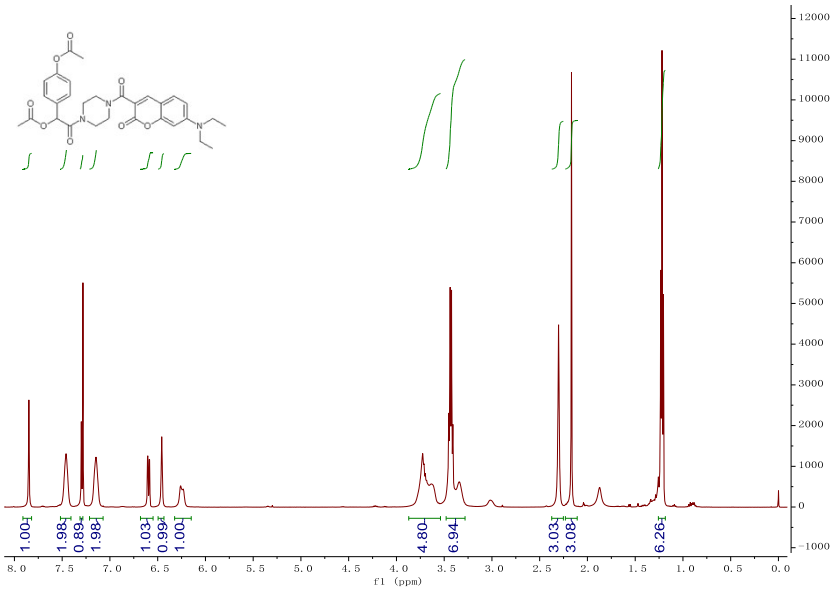


Fig. S22 ^{1}H-NMR of DHA-Blue

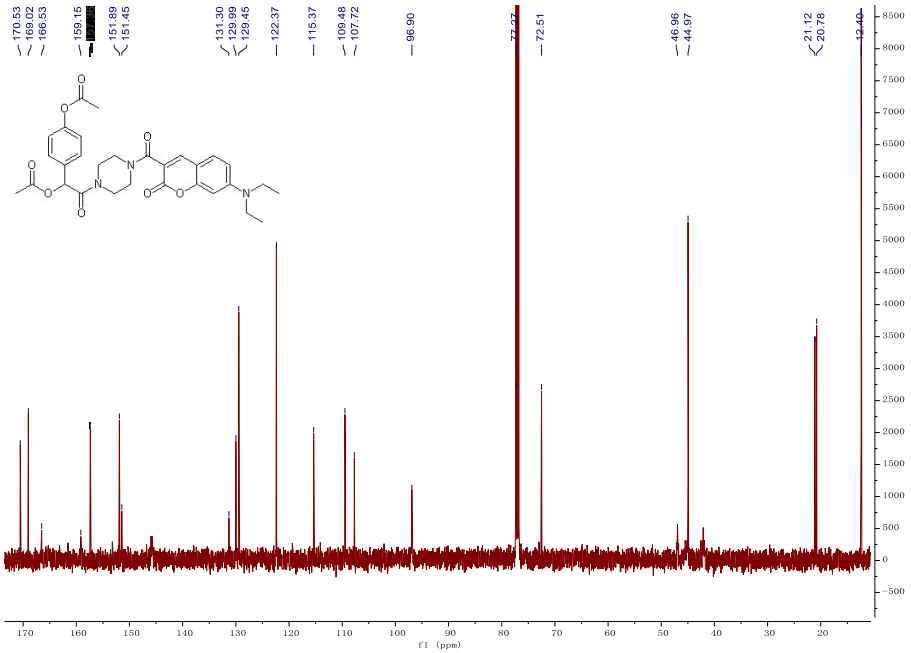


Fig. S23 ^{13}C-NMR of DHA-Blue

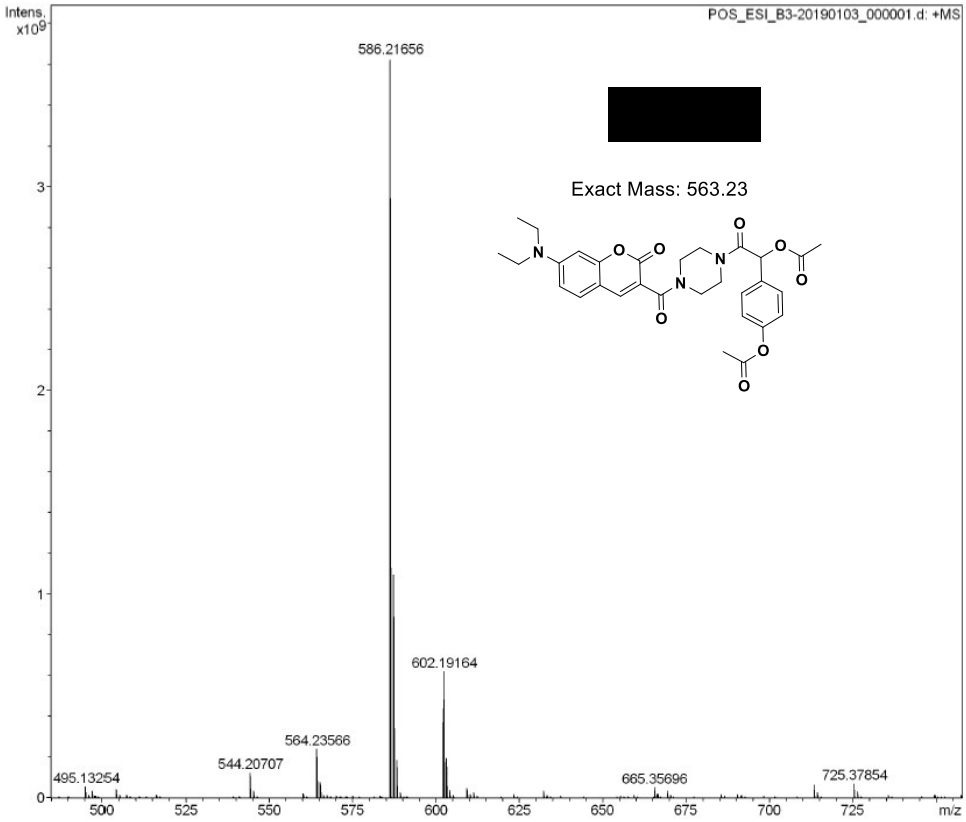


Fig. S24 HRMS of DHA-Blue

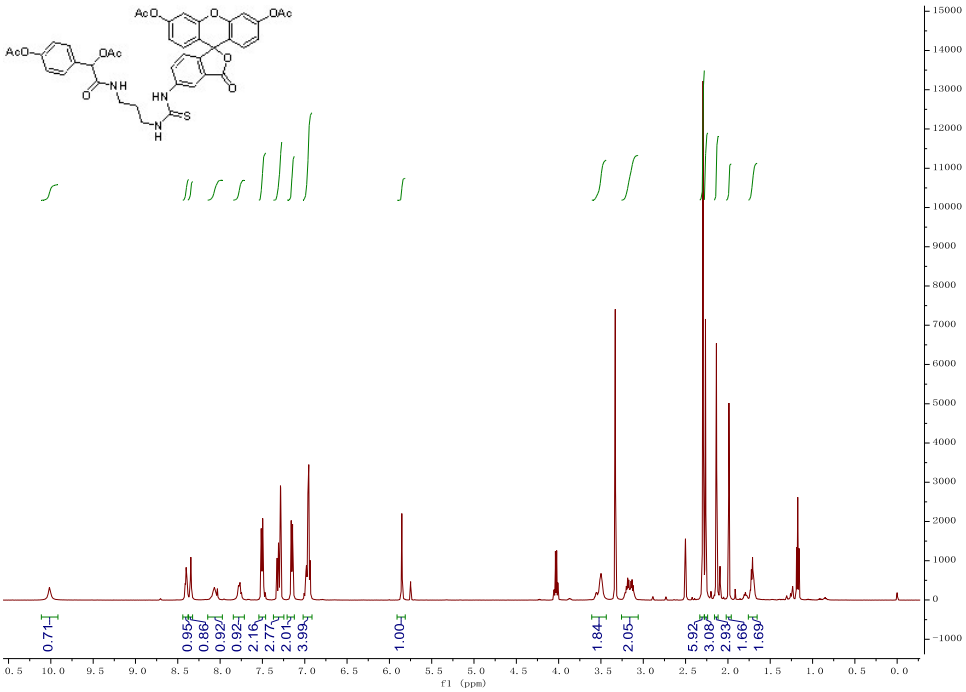


Fig. S25 ^{1}H-NMR of DHA-Green

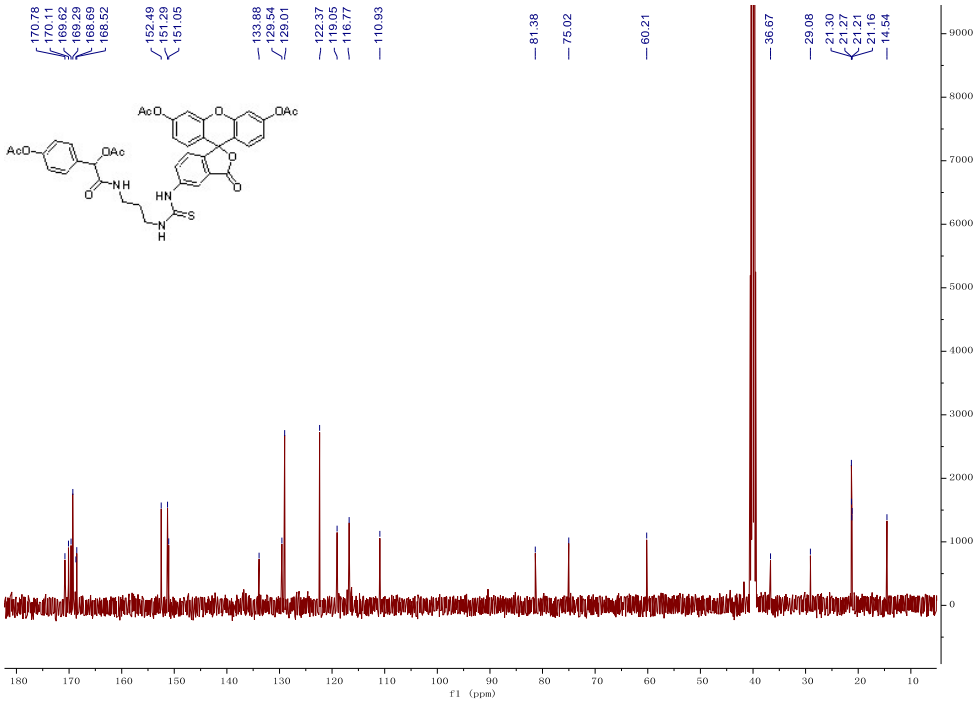


Fig. S26 ^{13}C-NMR of DHA-Green

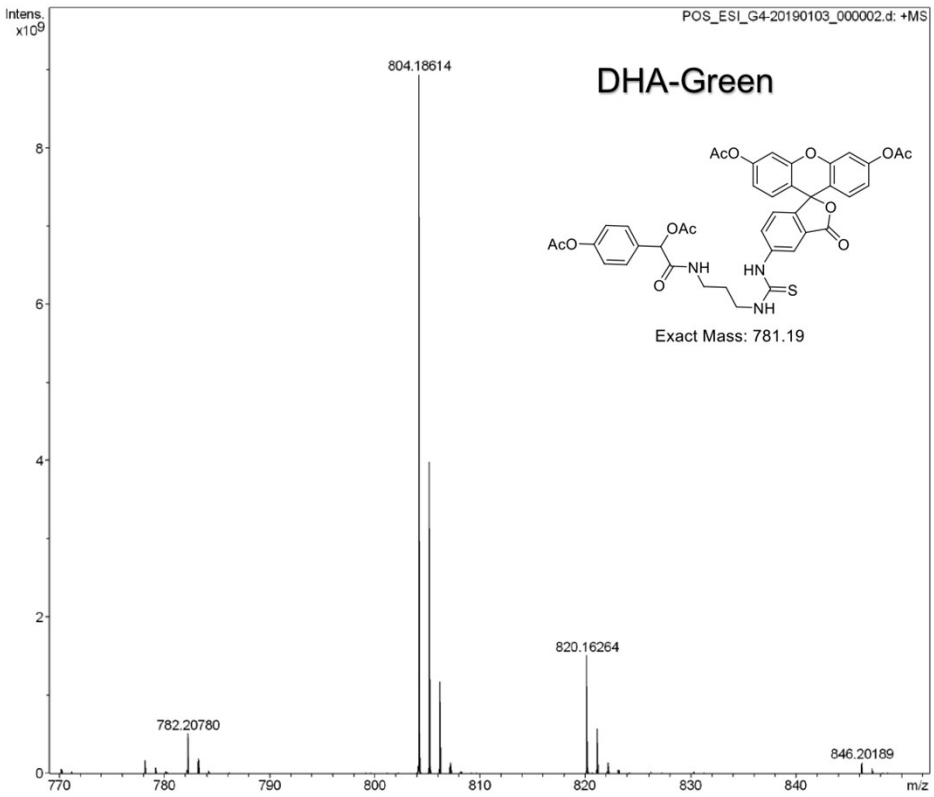


Fig. S27 HRMS of DHA-Green